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U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S OR COUNSEL MEMBER

## TRANSMITTAL LETTER TO THE UNITED STATES

1038-1003 MIS:jb

DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

09/462816

INTERNATIONAL APPLICATION NO.  
PCT/CA98/00697INTERNATIONAL FILING DATE  
16 July 1998PRIORITY DATE CLAIMED  
18 July 1997

## TITLE OF INVENTION

NUCLEIC ACID VACCINES ENCODING G PROTEIN OF RESPIRATORY SYNCYTIAL VIRUS

## APPLICANT(S) FOR DO/EO/US

Xiaomao Li; Suryaprakash Sambhara; and Michel H. Klein

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1.  This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2.  This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3.  This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4.  A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5.  A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
  - a.  is transmitted herewith (required only if not transmitted by the International Bureau).
  - b.  has been transmitted by the International Bureau.
  - c.  is not required, as the application was filed in the United States Receiving Office (RO/US).
6.  A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7.  A copy of the International Search Report (PCT/ISA/210).
8.  Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
  - a.  are transmitted herewith (required only if not transmitted by the International Bureau).
  - b.  have been transmitted by the International Bureau.
  - c.  have not been made; however, the time limit for making such amendments has NOT expired.
  - d.  have not been made and will not be made.
9.  A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10.  An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). -unsigned copy
11.  A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12.  A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

## Items 13 to 18 below concern document(s) or information included:

13.  An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14.  An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15.  A **FIRST** preliminary amendment.  
A **SECOND** or **SUBSEQUENT** preliminary amendment.
16.  A substitute specification.
17.  A change of power of attorney and/or address letter.
18.  Certificate of Mailing by Express Mail
19.  Other items or information:

Sequence Listing on computer-readable and on printed paper forms.

It is hereby stated that the Sequence Listing contained on the computer disk is the same as the Sequence Listing in printed paper form.

U.S. APPLICATION NO. (IF KNOWN) 37 CFR  
091462816INTERNATIONAL APPLICATION NO.  
PCT/CA98/00697ATTORNEY'S DOCKET NUMBER  
1038-1003 MIS:jb

20. The following fees are submitted:

**BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :**

<input type="checkbox"/> Search Report has been prepared by the EPO or JPO .....	\$840.00
<input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) .....	\$670.00
<input type="checkbox"/> No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) .....	\$760.00
<input checked="" type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO .....	\$970.00
<input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) .....	\$96.00

**CALCULATIONS PTO USE ONLY****ENTER APPROPRIATE BASIC FEE AMOUNT =**

\$970.00

Surcharge of \$130.00 for furnishing the oath or declaration later than  20  30 months from the earliest claimed priority date (37 CFR 1.492 (e)).

\$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	48 - 20 =	28	x \$18.00	\$504.00
Independent claims	8 - 3 =	5	x \$78.00	\$390.00
Multiple Dependent Claims (check if applicable).			<input type="checkbox"/>	\$0.00
<b>TOTAL OF ABOVE CALCULATIONS</b>		=		<b>\$1,864.00</b>
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable).			<input type="checkbox"/>	\$0.00
<b>SUBTOTAL</b>		=		<b>\$1,864.00</b>
Processing fee of \$130.00 for furnishing the English translation later than 20 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			<input type="checkbox"/>	\$0.00
<b>TOTAL NATIONAL FEE</b>		=		<b>\$1,864.00</b>
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).			<input type="checkbox"/>	\$0.00
<b>TOTAL FEES ENCLOSED</b>		=		<b>\$1,864.00</b>
			<b>Amount to be: refunded</b>	\$
			<b>charged</b>	\$

- A check in the amount of **\$1,864.00** to cover the above fees is enclosed.
- Please charge my Deposit Account No. **19-2253** in the amount of **A duplicate copy of this sheet is enclosed.** to cover the above fees.
- The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **19-2253** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

Michael I. Stewart

NAME

24,973

REGISTRATION NUMBER

January 13, 2000

DATE

09/462816

430 Rec'd PCT/PTO 14 JAN 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Our Ref: 1038-1003 MIS:jb

In re National Phase of International Application

No.: PCT/CA98/00697

International

Filing Date: July 16, 1998

Applicant: Xiaomao Li, et al.

Title: NUCLEIC ACID VACCINES ENCODING G PROTEIN OF  
RESPIRATORY SYNCYTIAL VIRUS

PRELIMINARY AMENDMENT

The Commissioner of Patents  
and Trademarks,

Washington, D.C. 20231,

U. S. A.

Dear Sir:

Please amend this application in the following manner:

In the Disclosure:

Before the first line of the specification, add the following:

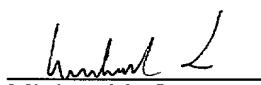
" REFERENCE TO RELATED APPLICATIONS

This application is a national phase application under 35 U.S.C. 371 of  
PCT/CA98/00697."

REMARKS

The specification has been amended on page 1 to reflect that this  
application is a U.S. National Phase filing under 35 U.S.C. 371 of PCT/CA98/00697.

Respectfully submitted,

  
\_\_\_\_\_  
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Date: January 13, 2000

TITLE OF INVENTIONNUCLEIC ACID VACCINES ENCODING G PROTEIN OF  
RESPIRATORY SYNCYTIAL VIRUS

5

FIELD OF INVENTION

The present invention is related to the field of respiratory syncytial virus (RSV) vaccines and is particularly concerned with vaccines comprising nucleic acid sequences encoding the attachment (G) protein of RSV.

BACKGROUND OF INVENTION

Respiratory syncytial virus (RSV), a negative-strand RNA virus belonging to the Paramyxoviridae family of viruses, is the major viral pathogen responsible for bronchiolitis and pneumonia in infants and young children (ref. 1 - Throughout this application, various references are referred to in parenthesis to more fully describe the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately preceding the claims. The disclosures of these references are hereby incorporated by reference into the present disclosure). Acute respiratory tract infections caused by RSV result in approximately 90,000 hospitalizations and 4,500 deaths per year in the United States (ref. 2). Medical care costs due to RSV infection are greater than \$340 M annually in the United States alone (ref. 3). There is currently no licensed vaccine against RSV. The main approaches for developing an RSV vaccine have included inactivated virus, live-attenuated viruses and subunit vaccines.

A protective immune response against RSV is thought to require the induction of neutralizing antibodies against the surface fusion (F) and attachment (G) glycoproteins (ref. 4). In addition, cytotoxic T lymphocytes (CTL) responses are involved in viral clearance. The F protein is conserved amongst the RSV A

and B subgroups.

The G protein (33 kDa) of RSV is heavily O-glycosylated giving rise to a glycoprotein of apparent molecular weight of 90 kDa (ref. 5). Two broad subtypes of RS virus have been defined: A and B (ref. 6). The major antigenic differences between these subtypes are found in the G glycoprotein (refs. 3, 7).

The use of RSV proteins as vaccines may have obstacles. Parenterally administered vaccine candidates have so far proven to be poorly immunogenic with regard to the induction of neutralizing antibodies in seronegative chimpanzees. The serum antibody response induced by these antigens may be further diminished in the presence of passively acquired antibodies, such as the transplacentally acquired maternal antibodies which most young infants possess. A subunit vaccine candidate for RSV consisting of purified fusion (F) glycoprotein from RSV infected cell cultures and purified by immunoaffinity or ion-exchange chromatography has been described (ref. 8). Parenteral immunization of seronegative or seropositive chimpanzees with this preparation was performed and three doses of 50 µg were required in seronegative animals to induce an RSV serum neutralizing titre of approximately 1:50. Upon subsequent challenge of these animals with wild-type RSV, no effect of immunization on virus shedding or clinical disease could be detected in the upper respiratory tract. The effect of immunization with this vaccine on virus shedding in the lower respiratory tract was not investigated, although this is the site where the serum antibody induced by parenteral immunization may be expected to have its greatest effect. Safety and immunogenicity studies have been performed in a small number of seropositive individuals. The vaccine was found to be safe in seropositive children and in three

seronegative children (all > 2.4 years of age). The effects of immunization on lower respiratory tract disease could not be determined because of the small number of children immunized. One immunizing dose in 5 seropositive children induced a 4-fold increase in virus neutralizing antibody titres in 40 to 60% of the vaccinees. Thus, insufficient information is available from these small studies to evaluate the efficacy of this vaccine against RSV-induced disease. A further 10 problem facing subunit RSV vaccines is the possibility that inoculation of seronegative subjects with immunogenic preparations might result in disease enhancement. In the 1960's, vaccination of infants with a formalin-inactivated RSV preparation (FI-RSV) resulted 15 in enhanced lung disease upon subsequent exposure to live virus, also referred to as immunopotentiation (refs. 9, 10). These vaccinees developed strong serological responses, but were not protected against infection and some developed severe, occasionally fatal 20 respiratory tract disease upon natural infection. Although precise mechanisms remain unknown, it has been suggested that this form of immune enhancement might reflect either structural alterations of RSV antigens (ref. 11), residual serum and/or cellular contaminants 25 (ref. 12), a specific property of the viral attachment (G) protein (refs. 13,14) or an imbalanced cell-mediated immune response (refs. 13,15). It has been demonstrated that the FI-RSV vaccine induced a TH2-type immune response in mice whereas immunization with live RSV, 30 which does not cause immunopotentiation, elicits a TH1 response (ref.15).

In some studies, the immune response to immunization with a synthetic RSV FG fusion protein resulted in disease enhancement in rodents resembling 35 that induced by a formalin-inactivated RSV vaccine.

Immunization of mice with a recombinant vaccinia virus expressing the RSV G protein resulted in G-specific T cell responses in the lungs which are exclusively recruited from the CD4+T cell sublineage and are 5 strongly Th2-biased. G-specific T cells induce lung haemorrhage, pulmonary neutrophil recruitment (shock lung), intense pulmonary eosinophilia, and sometimes death in the adoptively transferred murine recipients (ref. 14). The association of immunization with disease 10 enhancement using certain vaccine preparations including non-replicating antigens suggests caution in their use as vaccines in seronegative humans.

Live attenuated vaccines against disease caused by RSV may be promising for two main reasons. Firstly, 15 infection by a live vaccine virus induces a balanced immune response comprising mucosal and serum antibodies and cytotoxic T-lymphocytes. Secondly, infection of infants with live attenuated vaccine candidates or naturally acquired wild-type virus is not associated 20 with enhanced disease upon subsequent natural reinfection. It will be challenging to produce live attenuated vaccines that are immunogenic for younger infants who possess maternal virus-neutralizing antibodies and yet are attenuated for seronegative 25 infants greater than or equal to 6 months of age. Attenuated live virus vaccines also have the risks of residual virulence and genetic instability.

Injection of plasmid DNA containing sequences encoding a foreign protein has been shown to result in 30 expression of the foreign protein and the induction of antibody and cytotoxic T-lymphocyte (CTL) responses to the antigen in a number of studies (see, for example, refs. 16, 17, 18). The use of plasmid DNA inoculation to express viral proteins for the purpose of 35 immunization may offer several advantages over the

strategies summarized above. Firstly, DNA encoding a viral antigen can be introduced in the presence of antibody to the virus itself, without loss of potency due to neutralization of virus by the antibodies.

5 Secondly, the antigen expressed *in vivo* should exhibit a native conformation and the appropriate glycosylation. Therefore, the antigen should induce an antibody response similar to that induced by the antigen present in the wild-type virus infection. In contrast, some

10 processes used in purification of proteins can induce conformational changes which may result in the loss of immunogenicity of protective epitopes and possibly immunopotentiation. Thirdly, the expression of proteins from injected plasmid DNAs can be detected *in vivo* for a

15 considerably longer period of time than that in virus-infected cells, and this has the theoretical advantage of prolonged cytotoxic T-cell induction and enhanced antibody responses. Fourthly, *in vivo* expression of antigen may provide protection without the need for an

20 extrinsic adjuvant.

The ability to immunize against disease caused by RSV by administration of a DNA molecule encoding an RSV G protein was unknown before the present invention. In particular, the efficacy of immunization against RSV

25 induced disease using a gene encoding a secreted form of the RSV G protein was unknown. Infection with RSV leads to serious disease. It would be useful and desirable to provide isolated genes encoding RSV G protein and non-replicating vectors, including plasmid vectors, for *in*

30 *vivo* administration and for use in immunogenic preparations, including vaccines, for protection against disease caused by RSV and for the generation of diagnostic reagents and kits. In particular, it would be desirable to provide vaccines that are immunogenic

35 and protective in humans, including seronegative

infants, that do not cause disease enhancement (immunopotentiation).

SUMMARY OF INVENTION

The present invention relates to a method of 5 immunizing a host against disease caused by respiratory syncytial virus, to non-replicating vectors containing nucleic acid molecules used in immunogenic compositions for such purpose, and to diagnostic procedures utilizing the vectors and nucleic acid molecules. In particular, 10 the present invention is directed towards the provision of nucleic acid vaccines encoding the G protein of respiratory syncytial virus.

In accordance with one aspect of the invention, there is provided an immunogenic composition for *in vivo* 15 administration to a host for the generation in the host of protective antibodies to respiratory syncytial virus (RSV) G protein, comprising a non-replicating vector comprising:

a first nucleotide sequence encoding a RSV G 20 protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

25 a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host, and

a pharmaceutically-acceptable carrier therefor.

30 The first nucleotide sequence may be that which encodes a full-length RSV G protein. The first nucleotide sequence may comprise the nucleotide sequence shown in Figure 2 (SEQ. ID No: 1) or encode a full length RSV G protein having the amino acid sequence 35 shown in Figure 2 (SEQ. ID no: 2).

Alternatively, the first nucleotide sequence may be that which encodes an RSV G protein from which the transmembrane coding sequence and sequences upstream thereof are absent. The first nucleotide sequence 5 encoding the truncated RSV G protein may comprise the nucleotide sequence shown in Figure 3 (SEQ. ID no: 3) or may comprise a nucleotide sequence encoding the truncated RSV G protein having the amino acid sequence shown in Figure 3 (SEQ ID no: 4). The lack of 10 expression of the transmembrane region results in a secreted form of the RSV G protein.

The non-replicating vector may further comprise a heterologous signal peptide encoding nucleotide sequence immediately upstream of the 5'-terminus of the first 15 nucleotide sequence. The signal peptide encoding sequence may encode the signal peptide of human tissue plasminogen activator.

The promoter sequence may be an immediate early cytomegalovirus (CMV) promoter. The second nucleotide 20 sequence may comprise the human cytomegalovirus Intron A.

The non-replicating vector generally is a plasmid vector. Plasmid vectors encoding the G protein and included in the immunogenic composition provided by this 25 aspect of the invention may specifically be pXL5 or pXL6, constructed and having their characterizing elements, as seen in Figures 4 or 5, respectively.

In accordance with a further aspect of the present invention, there is provided a method of immunizing a 30 host against disease caused by infection with respiratory syncytial virus (RSV), which comprises administering to the host an effective amount of a non-replicating vector comprising:

a first nucleotide sequence encoding an RSV G 35 protein or a RSV G protein fragment that generates

antibodies that specifically react with RSV G protein,  
a promoter sequence operatively coupled to said  
first nucleotide sequence for expression of said RSV G  
protein in the host, and

- 5 a second nucleotide sequence located between said  
first nucleotide sequence and said promoter sequence to  
increase expression of said RSV G protein *in vivo* from  
said vector in the host.

The immunization method may be effected to induce a  
10 balanced Th1/Th2 immune response.

The present invention also includes a novel method  
of using a gene encoding respiratory syncytial virus  
(RSV) G protein or a RSV G protein fragment that  
generates antibodies that specifically react with RSV G  
15 protein, to protect a host against disease caused by  
infection with respiratory syncytial virus, which  
comprises:

- isolating the gene;  
operatively linking the gene to at least one  
20 control sequence to produce a non-replicating vector,  
said control sequence directing expression of the RSV G  
protein when said vector is introduced into a host to  
produce an immune response to the RSV G protein, and  
introducing the vector into the host.

25 The procedure provided in accordance with this aspect of  
the invention may further include the step of:

operatively linking the gene to an immunoprotection  
enhancing sequence to produce an enhanced  
immunoprotection by the RSV G protein in the host,  
30 preferably by introducing the immunoprotection enhancing  
sequence between the control sequence and the gene,  
including introducing immunostimulatory CpG sequences in  
the vector.

In addition, the present invention includes a  
35 method of producing a vaccine for protection of a host

against disease caused by infection with respiratory syncytial virus (RSV), which comprises:

isolating a first nucleotide sequence encoding an RSV G protein or a RSV G protein fragment that generates 5 antibodies that specifically react with RSV G protein,

operatively linking the first nucleotide sequence to at least one control sequence to produce a non-replicating vector, the control sequence directing expression of the RSV G protein when introduced into a 10 host to produce an immune response to the RSV G protein when expressed *in vivo* from the vector in a host,

operatively linking the first nucleotide sequence to a second nucleotide sequence to increase expression of the RSV G protein *in vivo* from the vector in a host, 15 and

formulating the vector as a vaccine for *in vivo* administration.

The vector may be a plasmid vector selected from pXL5 and pXL6. The invention further includes a vaccine 20 for administration to a host, including a human host, produced by this method.

As noted previously, the vectors provided herein are useful in diagnostic applications. In a further aspect of the invention, therefore, there is provided a 25 method of determining the presence of a respiratory syncytial virus (RSV) G protein in a sample, comprising the steps of:

(a) immunizing a host with a non-replicating vector to produce antibodies specific for the RSV G 30 protein, the non-replicating vector comprising a first nucleotide sequence encoding an RSV G protein or an RSV G protein fragment that generates antibodies that specifically react with RSV G protein, a promoter sequence operatively coupled to 35 the first nucleotide sequence for expression of the

RSV G protein in the host and a second nucleotide sequence located between the first nucleotide sequence and the promoter sequence to increase expression of the RSV G protein *in vivo* from the vector in the host;

- 5                   (b) isolating the RSV G protein-specific antibodies;
- 10                 (c) contacting the sample with the isolated antibodies to produce complexes comprising any RSV G protein present in the sample and the RSV G protein-specific antibodies; and
- 15                 (d) determining production of the complexes.

The non-replicating vector employed to elicit the antibodies may be a plasmid vector pXL5 or pXL6.

15                 The invention also includes a diagnostic kit for detecting the presence of a respiratory syncytial virus (RSV) G protein in a sample, comprising:

- 20                 (a) a non-replicating vector capable of generating antibodies specific for the RSV G protein when administered to a host, said non-replicating vector comprises a first nucleotide sequence encoding an RSV G protein or an RSV G protein fragment that generates antibodies that specifically react with RSV G protein, a promoter sequence operatively coupled to the first nucleotide sequence for expression of the RSV G protein in a host, and a second nucleotide sequence located between the first nucleotide sequence and the promoter sequence to increase expression of the RSV G protein *in vivo* from the vector in the host;
- 25                 (b) isolation means to isolate the RSV G protein specific antibodies;
- 30                 (c) contacting means to contact the isolated RSV G protein-specific antibodies with the sample to produce a complex comprising any RSV G protein

present in the sample and RSV G protein specific antibodies; and

(d) identifying means to determine production of the complex.

5 The present invention further is directed to a method for producing antibodies specific for a G protein of a respiratory syncytial virus (RSV) comprising:

10 (a) immunizing a host with an effective amount of a non-replicating vector to produce RSV G-specific antibodies, said non-replicating vector comprising:

a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

20 a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein in vivo from said vector in the host; and

(b) isolating the RSV G specific antibodies from the host.

25 The present invention is also directed to a method for producing monoclonal antibodies specific for a G protein of respiratory syncytial virus (RSV), comprising the steps of:

30 (a) constructing a vector comprising a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein, a promoter sequence operatively coupled to the first nucleotide sequence for expression of the RSV G protein in the host and a second nucleotide

sequence located between the first nucleotide sequence and the promoter sequence to increase expression of the RSV G protein when *in vivo* from the vector in a host;

- 5                 (b) administering the vector to at least one mouse to produce at least one immunized mouse;
- 10                 (c) removing B-lymphocytes from the at least one immunized mouse;
- (d) fusing the B-lymphocytes from the at least one immunized mouse with myeloma cells, thereby producing hybridomas;
- (e) cloning the hybridomas;
- (f) selecting clones which produce anti-RSV G protein antibody;
- 15                 (g) culturing the anti-RSV G protein antibody-producing clones; and
- (h) isolating anti-RSV G protein monoclonal antibodies.

Such monoclonal antibodies may be used to purify RSV G protein from virus.

In this application, the term "RSV G protein" is used to define a full-length RSV G protein, such proteins having variations in their amino acid sequences including those naturally occurring in various strains 25 of RSV, a secreted form of RSV G protein lacking a transmembrane region, as well as functional analogs of the RSV G protein. In this application, a first protein is a "functional analog" of a second protein if the first protein is immunologically related to and/or has 30 the same function as the second protein. The functional analog may be, for example, an immunologically-active fragment of the protein or an immunologically-active substitution, addition or deletion mutant thereof.

BRIEF DESCRIPTION OF THE FIGURES

The present invention will be further understood from the following General Description and Examples with reference to the Figures of the accompanying drawings,

5 in which:

Figure 1 illustrates a restriction map of the gene encoding a G protein of respiratory syncytial virus (RSV);

10 Figure 2 illustrates the nucleotide sequence of a gene encoding a membrane bound form of the G protein of respiratory syncytial virus (SEQ ID No: 1) as well as the amino acid sequence of the RSV G protein encoded thereby (SEQ ID No: 2);

15 Figure 3 illustrates the nucleotide sequence of a gene encoding the secreted form of the RSV G protein lacking the transmembrane domain (SEQ ID No: 3) as well as the amino acid sequence of a truncated RSV G protein lacking the transmembrane domain encoded thereby (SEQ ID No: 4);

20 Figure 4 shows the construction of plasmid pXL5 containing a gene encoding a full-length membrane attached form of the RSV G protein and containing the CMV Intron A sequence;

25 Figure 5 shows the construction of plasmid pXL6 containing a gene encoding a secreted form of the RSV G protein lacking the transmembrane domain and containing the CMV Intron A sequence as well as a nucleotide sequence encoding a signal peptide of the human tissue plasminogen activator (TPA);

30 Figure 6 shows the nucleotide sequence for the plasmid VR-1012 (SEQ ID No. 5);

Figure 7 shows the nucleotide sequence for the 5' untranslated region and the signal peptide of the human tissue plasminogen activator (TPA) (SEQ. ID no: 6) and

35 Figure 8 shows the lung cytokine expression profile

in DNA immunized mice after RSV challenge.

GENERAL DESCRIPTION OF INVENTION

As described above, the present invention relates generally to polynucleotide, including DNA, immunization 5 to obtain protection against infection by respiratory syncytial virus (RSV) and to diagnostic procedures using particular non-replicating vectors. In the present invention, several recombinant plasmid vectors were constructed to contain a nucleotide sequence encoding an 10 RSV G protein.

The nucleotide sequence of the full length RSV G gene is shown in Figure 2 (SEQ ID No: 1). Certain constructs provided herein include the nucleotide sequence encoding the full-length RSV G (SEQ ID No: 2) 15 protein while others include an RSV G gene modified by deletion of the transmembrane coding sequence and nucleotides upstream thereof (see Figure 3, SEQ ID No: 3), to produce a secreted or truncated RSV G protein lacking the transmembrane domain (SEQ ID No. 4).

20 The nucleotide sequence encoding the RSV G protein is operatively coupled to a promoter sequence for expression of the encoded RSV G protein *in vivo*. The promoter sequence may be the human immediately early cytomegalovirus (CMV) promoter. This promoter is 25 described in ref. 19. Any other convenient promoter may be used, including constitutive promoters, such as, the Rous Sarcoma Virus LTRs, and inducible promoters, such as the metallothionein promoter, and tissue specific promoters.

30 The non-replicating vectors provided herein, when administered to an animal in the form of an immunogenic composition with a pharmaceutically-acceptable carrier, effect *in vivo* RSV G protein expression, as demonstrated by an antibody response in the animal to which it is 35 administered. Such antibodies may be used herein in the

detection of RSV protein in a sample, as described in more detail below. The administration of the non-replicating vectors, specifically plasmids pXL5 and pXL6, produced anti-G antibodies, virus neutralizing 5 antibodies, a balanced Th1/Th2 response in the lungs post viral challenge and conferred protection in mice against live RSV infection, as seen from the Examples below.

The recombinant vector also may include a second 10 nucleotide sequence located adjacent the RSV G protein encoding nucleotide sequence to enhance the immunoprotective ability of the RSV G protein when expressed *in vivo* in a host. Such enhancement may be provided by increased *in vivo* expression, for example, 15 by increased mRNA stability, enhanced transcription and/or translation. This additional sequence generally is located between the promoter sequence and the RSV G protein-encoding sequence. This enhancement sequence may comprise the immediate early cytomegalovirus Intron 20 A sequence.

The non-replicating vector provided herein may also comprise an additional nucleotide sequence encoding a further antigen from RSV, an antigen from at least one other pathogen or at least one immunomodulating agent, 25 such as a cytokine. Such vector may contain the additional nucleotide sequence in a chimeric or a bicistronic structure. Alternatively, vectors containing the additional nucleotide sequence may be separately constructed and coadministered to a host, 30 along with the non-replicating vectors provided herein.

The non-replicating vector may further comprise a nucleotide sequence encoding a heterologous viral or eukaryotic signal peptide, such as the human tissue plasminogen activator (TPA) signal peptide, in place of 35 the endogenous signal peptide for the truncated RSV G

protein. Such nucleotide sequence may be located immediately upstream of the RSV G encoding sequence in the vector.

The immunogenicity of the non-replicating DNA vectors may be enhanced by inserting immunostimulatory CpG sequences in the vector.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis and treatment of RSV infections. A further non-limiting discussion of such uses is further presented below.

#### 1. Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as vaccines, may be prepared from the RSV G genes and vectors as disclosed herein. The vaccine elicits an immune response in an animal which includes the production of anti-RSV G antibodies. Immunogenic compositions, including vaccines, containing the nucleic acid may be prepared as injectables, in physiologically-acceptable liquid solutions or emulsions for polynucleotide administration. The nucleic acid may be associated with liposomes, such as lecithin liposomes or other liposomes known in the art, as a nucleic acid liposome (for example, as described in WO 9324640, ref. 20) or the nucleic acid may be associated with an adjuvant, as described in more detail below. Liposomes comprising cationic lipids interact spontaneously and rapidly with polyanions, such as DNA and RNA, resulting 30 in liposome/nucleic acid complexes that capture up to 100% of the polynucleotide. In addition, the polycationic complexes fuse with cell membranes, resulting in an intracellular delivery of polynucleotide that bypasses the degradative enzymes of the lysosomal 35 compartment. Published PCT application WO 94/27435

describes compositions for genetic immunization comprising cationic lipids and polynucleotides. Agents which assist in the cellular uptake of nucleic acid, such as calcium ions, viral proteins and other 5 transfection facilitating agents, may advantageously be used.

Polynucleotide immunogenic preparations may also be formulated as microcapsules, including biodegradable time-release particles. Thus, U.S. Patent 5,151,264 10 describes a particulate carrier of a phospholipid/glycolipid/polysaccharide nature that has been termed Bio Vecteurs Supra Moléculaires (BVSM). The particulate carriers are intended to transport a variety of molecules having biological activity in one of the 15 layers thereof.

U.S. Patent 5,075,109 describes encapsulation of the antigens trinitrophenylated keyhole limpet hemocyanin and staphylococcal enterotoxin B in 50:50 poly (DL-lactideco-glycolide). Other polymers for 20 encapsulation are suggested, such as poly(glycolide), poly(DL-lactide-co-glycolide), copolyoxalates, polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides), polyorthoesters and poly(8-hydroxybutyric acid), and polyanhydrides.

25 Published PCT application WO 91/06282 describes a delivery vehicle comprising a plurality of bioadhesive microspheres and antigens. The microspheres being of starch, gelatin, dextran, collagen or albumin. This delivery vehicle is particularly intended for the uptake 30 of vaccine across the nasal mucosae. The delivery vehicle may additionally contain an absorption enhancer.

The RSV G gene containing non-replicating vectors may be mixed with pharmaceutically acceptable excipients which are compatible therewith. Such excipients may 35 include, water, saline, dextrose, glycerol, ethanol, and

combinations thereof. The immunogenic compositions and vaccines may further contain auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness thereof.

- 5 Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously, intravenously, intradermally or intramuscularly, possibly following pretreatment of the injection site with a local anesthetic. Alternatively, the immunogenic
- 10 compositions formed according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces. Thus, the immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral
- 15 (intragastric) routes. Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example, polyalkylene glycols or triglycerides. Oral
- 20 formulations may include normally employed incipients, such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate.

The immunogenic preparations and vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize the RSV G protein and antibodies thereto, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of about 1 µg

to about 2 mg of the RSV G gene-containing vectors. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations.

- 5 The dosage may also depend on the route of administration and will vary according to the size of the host. A vaccine which protects against only one pathogen is a monovalent vaccine. Vaccines which contain antigenic material of several pathogens are  
10 combined vaccines and also belong to the present invention. Such combined vaccines contain, for example, material from various pathogens or from various strains of the same pathogen, or from combinations of various pathogens.
- 15 Immunogenicity can be significantly improved if the vectors are co-administered with adjuvants, commonly used as 0.05 to 0.1 percent solution in phosphate-buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic  
20 themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen  
25 depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Thus, adjuvants have been  
30 identified that enhance the immune response to antigens. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively  
35 commonly referred to as alum) are routinely used as

adjuvants in human and veterinary vaccines.

A wide range of extrinsic adjuvants and other immunomodulating material can provoke potent immune responses to antigens. These include saponins complexed  
5 to membrane protein antigens to produce immune stimulating complexes (ISCOMS), pluronic polymers with mineral oil, killed mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as  
10 well as monophoryl lipid A, QS 21 and polyphosphazene.

In particular embodiments of the present invention, the non-replicating vector comprising a first nucleotide sequence encoding an G protein of RSV may be delivered in conjunction with a targeting molecule to target the  
15 vector to selected cells including cells of the immune system.

The immunogenicity of the non-replicating vector may be enhanced by coadministering plasmid DNA vectors expressing cytokines or chemokines or by coexpressing  
20 such molecules in a bis-cistronic or fusion construct.

The non-replicating vector may be delivered to the host by a variety of procedures, for example, Tang et al. (ref. 21) disclosed that introduction of gold microprojectiles coated with DNA encoding bovine growth  
25 hormone (BGH) into the skin of mice resulted in production of anti-BGH antibodies in the mice, while Furth et al. (ref. 22) showed that a jet injector could be used to transfect skin, muscle, fat and mammary tissues of living animals.

### 30 2. Immunoassays

The RSV G genes and vectors of the present invention are useful as immunogens for the generation of anti-G antibodies for use in immunoassays, including enzyme-linked immunosorbent assays (ELISA), RIAs and  
35 other non-enzyme linked antibody binding assays or

procedures known in the art. In ELISA assays, the non-replicating vector first is administered to a host to generate antibodies specific to the RSV G protein. These RSV G-specific antibodies are immobilized onto a selected surface, for example, a surface capable of binding the antibodies, such as the wells of a polystyrene microtiter plate. After washing to remove unadsorbed antibodies, a non-specific protein, such as a solution of bovine serum albumin (BSA) that is known to be antigenically neutral with regard to the test sample, may be bound to the selected surface. This allows for blocking of non-specific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of antisera onto the surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This procedure may include diluting the sample with diluents, such as solutions of BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to incubate for from about 2 to 4 hours, at temperatures such as of the order of about 20° to 37°C. Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution, such as PBS/Tween or a borate buffer. Following formation of specific immunocomplexes between the test sample and the bound RSV G specific antibodies, and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined.

#### BIOLOGICAL MATERIALS

Certain plasmids that contain the gene encoding the RSV G protein and referred to herein have been deposited

with the American Type Culture Collection (ATCC) located at 12301 Parklawn Drive, Rockville, Maryland, 20852, U.S.A., pursuant to the Budapest Treaty and prior to the filing of this application.

- 5 Samples of the deposited plasmids will become available to the public upon grant of a patent based upon this United States patent application and all restrictions on access to the deposits will be removed at that time. Samples of the deposited plasmids will be  
10 replaced if the depository is unable to dispense viable samples. The invention described and claimed herein is not to be limited in scope by plasmids deposited, since the deposited embodiment is intended only as an illustration of the invention. Any equivalent or  
15 similar plasmids that encode similar or equivalent antigens as described in this application are within the scope of the invention.

	<u>Plasmid</u>	<u>ATCC Designation</u>	<u>Date Deposited</u>
	pXL5	209143	July 16, 1997
20	pXL6	209144	July 16, 1997

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific  
25 Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although  
30 specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, protein biochemistry, and immunology used but not explicitly  
35 described in this disclosure and these Examples are

amply reported in the scientific literature and are well within the ability of those skilled in the art.

Example 1

This Example describes the construction of vectors 5 containing the RSV G gene.

Figure 1 shows a restriction map of the gene encoding the G protein of respiratory syncytial virus and Figure 2 shows the nucleotide sequence of the gene encoding the full-length RSV G protein (SEQ ID No: 1) 10 and the deduced amino acid sequence (SEQ ID No: 2).

Figure 3 shows the gene encoding the secreted RSV G protein (SEQ ID No: 3) and the deduced amino acid sequence (SEQ ID No: 4).

Plasmid pXL5 (Figure 4) was prepared for the 15 expression of the full-length RSV G protein as follows:

A recombinant Bluescript plasmid (RSV G12) containing the cDNA encoding the full-length G protein of a clinical RSV isolate (subgroup A) was used to construct vectors for RSV DNA-G immunization. RSV G12 20 was digested with AflIII and EcoRI and filled-in with the Klenow subunit of DNA polymerase. The resulting 1.23 kb fragment containing the coding sequence for the full-length G protein was gel-purified and ligated to VR-1012 (Vical) (Figure 6) previously linearized with 25 EcoRV. This procedure placed the RSV G cDNA downstream of the immediate-early cytomegalovirus (CMV) promoter and Intron A sequences of human cytomegalovirus (CMV) and upstream of the bovine growth hormone (BGH) poly-A site. The junctions of the cDNA fragments in the plasmid 30 construct were confirmed by sequencing analysis. The resulting plasmid was designated pXL5.

Plasmid pXL6 (Figure 5) was prepared for the expression of a secretory RSV G protein as follows:

RSV G12 was digested with EcoRI, filled-in with 35 Klenow and digested again with BamHI. The BamHI

cleavage resulted in the generation of a cDNA fragment encoding a RSV G protein with N-terminal truncation. This DNA segment was gel-purified and ligated in the presence of a pair of 11 mer oligodeoxynucleotides

5 (5'GATCCACTCAG 3') (SEQ ID no: 7)  
3' GTGAGTCCTAG 5' (SEQ ID no: 8)

to VR-1020 (Vical) previously digested with BglII, filled in with Klenow, digested again with BamHI and gel-purified. This procedure placed the truncated RSV G

10 cDNA (lacking the coding region for the N-terminal 91 amino acid residues including the transmembrane domain) downstream of the immediate-early CMV promoter and Intron A sequences of human CMV and upstream of the BGH poly-A site. In addition, there was the introduction of

15 approximately 100 bp of 5' untranslated region and the coding sequence for the signal peptide of human plasminogen activator protein (Figure 7) fused in frame to the N-terminus of the RSV G protein coding sequence downstream of the CMV promoter/Intron A sequences. The

20 junctions of the cDNA fragments in the plasmid construct were confirmed by sequencing analysis. The resulting plasmid was designated pXL6.

Example 2

This Example describes the immunization of mice.

25 Mice are susceptible to infection by RSV as described in ref. 24.

Plasmid DNA was purified through double CsCl centrifugations. For intramuscular (i.m.) immunization, tibialis anterior muscles of BALB/c mice (male, 6 to 8

30 week old) (Jackson Lab., Bar Harbor, ME, USA) were bilaterally injected with 2 x 50 $\mu$ g (1 $\mu$ g/ $\mu$ L in PBS) of either pXL5, pXL6 or V-1012. Five days prior to DNA injection, the muscles were treated with 2 x 50 $\mu$ L (10 $\mu$ M in PBS) of cardiotxin (Latoxan, France) to increase DNA

35 uptake and enhance immune responses, as reported by

Davis et al (ref. 23). The animals were boosted with the same dose of plasmid DNA 6 weeks and 13 weeks later, respectively. For intradermal (i.d.) immunization, 100 $\mu$ g of the plasmid DNA (2 $\mu$ g/ $\mu$ L in PBS) of were 5 injected at the base of the tail and boosted 6 weeks and 13 weeks later, respectively. Mice in the positive control group were immunized intranasally (i.n.) with 10<sup>6</sup> plaque forming units (pfu) of a clinical RSV strain of the A2 subtype grown in Hep2 cells kindly provided by 10 Dr. B. Graham (ref. 24).

Four weeks after the third immunization, mice were challenged intranasally with 10<sup>6</sup> pfu of the RSV A2 strain. Lungs were aseptically removed 4 days later, weighed and homogenized in 2 mL of complete culture 15 medium (ref. 25). The number of pfu in lung homogenates was determined in duplicate as previously described (ref. 26) using vaccine-quality Vero cells.

Example 3

This Example describes the immunogenicity and 20 protection by polynucleotide immunization.

Antisera obtained from immunized mice were analyzed for anti-RSV G IgG antibody titres using specific enzyme-linked immunosorbent assay (ELISA) and for RSV-specific plaque-reduction titres. ELISAs were performed 25 using 96-well plates coated with immunoaffinity-purified RSV G protein (50 ng/mL) and 2-fold serial dilutions of immune sera. A goat anti-mouse IgG antibody conjugated to alkaline phosphatase (Jackson ImmunoRes., Mississauga, Ontario, Canada) was used as secondary 30 antibody. Plaque reduction titres were determined according to Prince et al (ref. 26) using vaccine-quality Vero cells. Four-fold serial dilutions of immune sera were incubated with 50 pfu of the RSV Long strain (ATCC) in culture medium at 37°C for 1 hr in the 35 presence of 5% CO<sub>2</sub> and the mixtures were used to infect

Vero cells. Plaques were fixed with 80% methanol and developed 5 days later using a mouse anti-RSV F monoclonal IgG1 antibody and donkey anti-mouse IgG antibody conjugated to peroxidase (Jackson ImmunoRes.,  
5 Mississauga, Ontario, Canada). The RSV-specific plaque reduction titre was defined as the dilution of serum sample yielding 60% reduction in plaque number. Both ELISA and plaque reduction assays were performed in duplicate and data are expressed as the means of two  
10 determinations.

The results obtained are reproduced in Tables I and II below:

**Table I. Immunogenicity of DNA-G in BALB/c Mice**

<i>Immunogen Titre</i>	<i>Anti-RSV G IgG Titre (Log 2(titre/100))</i>			<i>RSV-Specific Plaque Reduction (Log 2 titre) 17 weeks</i>
	<i>6 weeks</i>	<i>10 weeks</i>	<i>17 weeks</i>	
VR-1012 (i.m.)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
pXL5 (i.m.)	3.10 ± 2.77	9.70 ± 1.06	8.60 ± 1.17	5.40 ± 1.65
pXL6 (i.m.)	5.78 ± 1.20	9.30 ± 0.82	8.89 ± 1.54	7.26 ± 0.82
pXL5 (i.d.)	1.50 ± 1.27	8.60 ± 1.43	8.30 ± 1.25	7.92 ± 0.59
pXL6 (i.d.)	3.70 ± 1.25	10.30 ± 1.06	9.44 ± 1.24	6.92 ± 0.94
RSV (i.n.)	6.83 ± 0.41	9.67 ± 0.52	9.83 ± 0.41	11.80 ± 0.08

**Table II. Immunoprotective Ability of DNA-G in BALB/c Mice**

<i>Immunogen</i>	<i>No. Mice</i>	<i>Mean Virus Lung Titre* (pfu/g lung) (Log 10 <math>\pm</math> SD)</i>	<i>No. Fully Protected Mice#</i>
VR-1012 (i.m.)	6	4.81 $\pm$ 0.01	0
pXL5 (i.m.)	6	0.29 $\pm$ 0.90	5
pXL6 (i.m.)	6	0.40 $\pm$ 1.20	5
pXL5 (i.d.)	6	0.30 $\pm$ 1.10	5
pXL6 (i.d.)	6	0.29 $\pm$ 0.90	5
RSV (i.n.)	6	0.00 $\pm$ 0.00	6

\*Sensitivity of the assay: 10<sup>1.96</sup> pfu/g lung.

# The term, fully protected mice, refers to animals with no detectable RSV in the lungs 4 days post viral challenge.

As seen in Table I, plasmids pXL5 and pXL6 were found to be immunogenic following either i.m. or i.d. immunization producing anti-G antibodies and virus neutralizing antibodies. In addition, as seen in Table II, the plasmids pXL5 and pXL6 protected immunized mice against primary RSV infection of the lower respiratory tract. The control vector produced no immune response and did not confer protection.

Example 4

This Example describes the determination of the local lung cytokine expression profile in mice immunized with pXL5 and pXL6 after RSV challenge.

BALB/c mice were immunized at 0 and 6 weeks with 100 $\mu$ g of pXL5 and 6, prepared as described in Example 1, and challenged with RSV i.n. at 10 weeks. Control animals were immunized with placebo PI-RSV and live RSV and challenged with RSV according to the same protocol. In addition, animals were immunized with pXL2, as described in copending United States Patent Application no. 08/476,397 filed June 7, 1995 (WO 96/40945) and challenged with RSV, also following the same protocol. Four days post viral challenge, lungs were removed from immunized mice and immediately frozen in liquid nitrogen. Total RNA was prepared from lungs homogenized in TRIzol/ $\beta$ -mercaptoethanol by chloroform extraction and isopropanol precipitation. Reverse transcriptase-polymerase chain reaction (RT-PCR) was then carried out on the RNA samples using either IL-4, IL-5 or IFN- $\gamma$  specific primers from CloneTech. The amplified products were then liquid-hybridized to cytokine-specific  $^{32}$ P-labeled probes from CloneTech, resolved on 5% polyacrylamide gels and quantitated by scanning of the radioactive signals in the gels. Three mouse lungs were removed from each treatment group and analyzed for lung cytokine expression for a minimum of two times. The

data is presented in Figure 8 and represents the means and standard deviations of these determinations.

As may be seen from the data presented in Figure 8:

- 5        1. Immunization with live RSV intranasally (i.n.) resulted in a balanced cytokine profile (IFN- $\gamma$ , IL-4 and IL-5), whereas that with FI-RSV intramuscularly (i.m.) resulted in a Th2 predominance (elevated IL-4 and IL-5). These results are similar to those reported in the literature.
- 10        2. Immunization with pXL5 or pXL6 via either the i.m. or intradermal (i.d.) route gave rise to a balanced cytokine profile similar to that with live RSV immunization.
- 15        3. The magnitude of the cytokine responses with i.m. pXL6 (RSV G) and pXL2 (RSV F) immunization using the construct expressing a secretory form of the protein (SEC) is significantly higher than that with live RSV immunization.
- 20        4. The magnitude of the cytokine response with pXL5 immunization using constructs expressing a full-length membrane-associated RSV G protein (MA) and i.d. pXL6 was somewhat higher than that with live RSV immunization.
- 25        5. The balanced local cytokine response observed with DNA-G immunization contrasts with that reported by Openshaw et al (ref. 13). Using a recombinant vaccinia virus expressing the G protein, these investigators reported a local Th2 response by analysis of bronchoalveolar lavage. The results herein, which were obtained through a monogenic approach, indicate that the Th2 response is not necessarily an intrinsic property of the G protein.

SUMMARY OF THE DISCLOSURE

In summary of this disclosure, the present invention provides certain novel non-replicating vectors containing genes encoding RSV G proteins, methods of immunization using such vectors and methods of diagnosis using such vectors. Modifications are possible within the scope of this invention.

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Article 34

CLAIMS

What we claim is:

1. An immunogenic composition for *in vivo* administration to a host for the generation in the host of protective antibodies to respiratory syncytial virus (RSV) G protein, comprising a vector that will not replicate when introduced into the host to be protected comprising:
  - a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,
  - a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and
  - a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host, and
  - a pharmaceutically-acceptable carrier therefor.
2. The composition of claim 1 wherein said first nucleotide sequence encodes a full-length RSV G protein.
3. The composition of claim 2 wherein said nucleotide sequence comprises the nucleotide sequence shown in Figure 2 (SEQ ID NO:1).
4. The composition of claim 2 wherein said first nucleotide sequence comprises the nucleotide sequence encoding a full length RSV G protein having the amino acid sequence shown in Figure 2 (SEQ ID NO:2).
5. The composition of claim 1 wherein said first nucleotide sequence encodes a RSV G protein from which the transmembrane coding sequence and sequences upstream thereto are absent.
6. The composition of claim 5 wherein said vector further comprises a heterologous signal peptide encoding nucleotide sequence immediately upstream of the 5'-terminus of said first nucleotide sequence.
- 30 7. The composition of claim 6 wherein said signal peptide encoding sequence encodes the signal peptide for human tissue plasminogen activator.

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8. The composition of claim 5 wherein said first nucleotide sequence comprises the nucleotide sequence shown in Figure 3 (SEQ ID NO:3).
9. The composition of claim 5 wherein said first nucleotide sequence 5 comprises a nucleotide sequence encoding a truncated RSV G protein having the amino acid sequence shown in Figure 3 (SEQ ID NO:4).
10. The composition of claim 1 wherein said promoter sequence is an immediate early cytomegalovirus promoter.
11. The composition of claim 1 wherein said second nucleotide sequence 10 is the human cytomegalovirus Intron A.
12. The composition of claim 1 wherein the vector is a plasmid vector.
13. The composition of claim 12 wherein the plasmid vector is pXL5 as shown in Figure 4.
14. The composition of claim 12 wherein the plasmid vector is pXL6 as 15 shown in Figure 5.
15. A method of immunizing a host against disease caused by infection with respiratory syncytial virus (RSV), which comprises administering to said host an effective amount of a vector that will not replicate when introduced into the host to be protected comprising:
- 20 a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,
- 25 a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and
- 25 a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host.
16. The method of claim 15 wherein said first nucleotide sequence encodes a full-length RSV G protein.
- 30 17. The method of claim 16 wherein said nucleotide sequence comprises the nucleotide sequence shown in Figure 2 (SEQ ID NO:1).

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18. The method of claim 16 wherein said first nucleotide sequence comprises the nucleotide sequence encoding a full length RSV G protein shown in Figure 2 (SEQ ID NO:2).
19. The method of claim 15 wherein said first nucleotide sequence encodes a RSV G protein from which the transmembrane coding sequence and sequences upstream thereto are absent.
20. The method of claim 19 wherein said vector further comprises a heterologous signal peptide encoding nucleotide sequences immediately upstream of the 5'-terminus of said first nucleotide sequence.
21. The method of claim 20 wherein said signal peptide encoding sequence encodes the signal peptide for human tissue plasminogen activator.
22. The method of claim 19 wherein said first nucleotide sequence comprises the nucleotide sequence shown in Figure 3 (SEQ ID NO:3).
23. The method of claim 19 wherein said first nucleotide sequence comprises a nucleotide sequence encoding a transverse RSV G protein shown in Figure 3 (SEQ ID NO:4).
24. The method of claim 15 wherein said promoter sequence is an immediate early cytomegalovirus promoter.
25. The method of claim 15 wherein said second nucleotide sequence is the human cytomegalovirus Intron A.
26. The method of claim 15 wherein the vector is a plasmid vector.
27. The method of claim 26 wherein said plasmid vector is pXL5 as shown in Figure 4.
28. The method of claim 26 wherein said vector is  $\phi$ XLG as shown in Figure 5.
29. The method of claim 15 wherein a balanced Th1/Th2 immune response is induced.
30. A method of using a gene encoding a respiratory syncytial virus (RSV) G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein, to produce an immune response in a host, which comprises:



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40. A method of producing a vaccine for protection of a host against disease caused by infection with respiratory syncytial virus (RSV), which comprises:

- isолating a first nucleotide sequence encoding a RSV G protein or a  
5 RSV G protein fragment that generates antibodies that specifically react with  
RSV G protein,

operatively linking said first nucleotide sequence to at least one control sequence to produce a vector that will not replicate when introduced into the host to be protected, the control sequence directing expression of  
10 said RSV G protein when introduced to a host to produce an immune response to said RSV G protein,

operatively linking said first nucleotide sequence to a second nucleotide sequence to increase expression of said RSV G protein *in vivo* from the vector in the host, and

- 15 formulating said vector as a vaccine for *in vivo* administration to a host.

41. The method of claim 40 wherein said vector is selected from group consisting of pXL5 and pXL6.

42. A vaccine produced by the method of claim 40.

- 20 43. A method of determining the presence of a respiratory syncytial virus (RSV) G protein in a sample, comprising the steps of:

(a) immunizing a host with a vector that will not replicate when introduced into the host to be protected to produce antibodies specific for the RSV G protein, said vector comprising:

- 25 a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

- 30 a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host,

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- (b) isolating the RSV G protein specific antibodies;
- (c) contacting the sample with the isolated antibodies to produce complexes comprising any RSV G protein present in a sample and said isolated RSV G protein-specific antibodies; and
- 5 (d) determining the production of the complexes.

44. The method of claim 43 wherein said vector is selected from the group consisting of pXL5 and pXL6.

45. A diagnostic kit for detecting the presence of a respiratory syncytial virus (RSV) G protein in a sample, comprising:

- 10 (a) a vector that will not replicate when introduced into the host to be protected capable of generating antibodies specific for the RSV G protein when administered to a host, the vector comprising:
  - a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,
  - 15 a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and
  - a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host;
- 20 (b) isolation means to isolate said RSV G protein-protein-specific antibodies;
- (c) contacting means to contact the isolated RSV G specific antibodies with the sample to produce a complex comprising any RSV G protein in the sample and RSV G protein specific antibodies, and
- 25 (d) identifying to determine production of the complex.

46. The diagnostic kit of claim 45 wherein said vector is selected from the group consisting of pXL5 and pXL6.

47. A method for producing antibodies specific for a G protein of respiratory syncytial virus (RSV) comprising:

40

(a) immunizing a host with an effective amount of a vector that will not replicate when introduced into the host to be protected to produce RSV G-specific antibodies, said vector comprising:

5 a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

10 a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host; and

(b) isolating the RSV G-specific antibodies from the host.

15 48. A method of producing monoclonal antibodies specific for a G protein of respiratory syncytial virus (RSV) comprising the steps of:

(a) constructing a vector that will not replicate when introduced into the host to be protected comprising:

20 a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

25 a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host;

(b) administering the vector to at least one mouse to produce at least one immunized mouse;

30 (c) removing B-lymphocytes from the at least one immunized mouse;

41

- (d) fusing the B- lymphocytes from the at least one immunized mouse with myeloma cells, thereby producing hybridomas;
- (e) cloning the hybridomas;
- 5 (f) selecting clones which produce anti-RSV G protein antibody;
- (g) culturing the anti-RSV G protein antibody-producing clones; and then
- (h) isolating anti-RSV G protein antibodies from the cultures.

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SEQUENCE LISTING

<110> LI, Xiaomao  
SAMBHARA, Suryaprakash  
KLEIN, Michel H.

<120> NUCLEIC ACID VACCINES ENCODING G PROTEIN OF RESPIRATORY  
SYNCTIAL VIRUS

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Leu Asn Leu Lys Ser Val Ala Gln Ile Thr Leu Ser Ile Leu Ala Met  
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 Ile Ile Ser Thr Ser Leu Ile Ile Thr Ala Ile Ile Phe Ile Ala Ser  
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 Ala Asn His Lys Val Thr Leu Thr Thr Ala Ile Ile Gln Asp Ala Thr  
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 Ser Gln Ile Lys Asn Thr Thr Pro Thr Tyr Leu Thr Gln Asp Pro Gln  
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 Leu Gly Ile Ser Phe Ser Asn Leu Ser Glu Ile Thr Ser Gln Thr Thr  
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 Thr Ile Leu Ala Ser Thr Thr Pro Gly Val Lys Ser Asn Leu Gln Pro  
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 Thr Thr Val Lys Thr Lys Asn Thr Thr Thr Gln Thr Gln Pro Ser  
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 Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn Lys Pro Asn  
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 Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys  
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 Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys  
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 Lys Pro Gly Lys Lys Thr Thr Lys Pro Thr Lys Lys Pro Thr Phe  
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 Lys Thr Thr Lys Lys Asp Leu Lys Pro Gln Thr Thr Lys Pro Lys Glu  
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 Val Pro Thr Thr Lys Pro Thr Glu Glu Pro Thr Ile Asn Thr Thr Lys  
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 Thr Asn Ile Thr Thr Leu Leu Thr Asn Asn Thr Thr Gly Asn Pro  
     245                       250                       255  
 Lys Leu Thr Ser Gln Met Glu Thr Phe His Ser Thr Ser Glu Gly  
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 Asn Leu Ser Pro Ser Gln Val Ser Thr Thr Ser Glu His Pro Ser Gln  
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 Pro Ser Ser Pro Pro Asn Thr Thr Arg Gln  
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 actacaaaac aacgccaaaa caaaccacca aacaaaccca ataatgattt tcacttcgaa 300  
 gtgttaact ttgtaccctg cagcatatgc agcaacaatc caacctgctg ggctatctgc 360  
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 ctgctcacca acaacaccac aggaaatcca aaactcacaa gtcaaattgaa aaccttccac 600  
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Ile Ser Phe Ser Asn Leu Ser Glu Ile Thr Ser Gln Thr Thr Ile  
 35 40 45

Leu Ala Ser Thr Thr Pro Gly Val Lys Ser Asn Leu Gln Pro Thr Thr  
 50 55 60

Val Lys Thr Lys Asn Thr Thr Gln Thr Gln Pro Ser Lys Pro  
 65 70 75 80

Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn Lys Pro Asn Asn Asp  
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Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Ser Asn  
 100 105 110

Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys Lys Pro  
 115 120 125

Gly Lys Lys Thr Thr Lys Pro Thr Lys Lys Pro Thr Phe Lys Thr  
 130 135 140

Thr Lys Lys Asp Leu Lys Pro Gln Thr Thr Lys Pro Lys Glu Val Pro  
 145 150 155 160

Thr Thr Lys Pro Thr Glu Pro Thr Ile Asn Thr Thr Lys Thr Asn  
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Ile Thr Thr Thr Leu Leu Thr Asn Asn Thr Thr Gly Asn Pro Lys Leu  
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Thr Ser Gln Met Glu Thr Phe His Ser Thr Ser Ser Glu Gly Asn Leu  
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 cgcccgac 188

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11

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## Restriction Map of the RSV G Gene

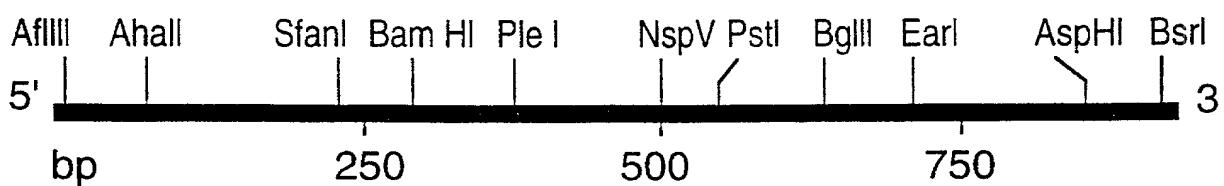


FIG.1

**FIG.2A**

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10	19	28	37	46	55
TGCAAC ATG TCC AAA AAC AAG GAC CAA CGC ACC GCT AAG ACA CTA GAA AAG ACC					
Met Ser Lys Asn Lys Asp Gln Arg Thr Ala Lys Thr Leu Glu Lys Thr					
64	73	82	91	100	109
TGG GAC ACT CTC AAT CAT TTA TTA TTC ATA TCA TCG GCC TTA TAT AAG TTA AAT					
Trp Asp Thr Leu Asn His Leu Leu Phe Ile Ser Ser Gly Leu Tyr Lys Leu Asn					
118	127	136	145	154	163
CTT AAA TCT GTT GCA CAA ATC ACA TTA TCC ATT CTG GCA ATG ATA ATC TCA ACT					
Leu Lys Ser Val Ala Gln Ile Thr Leu Ser Ile Leu Ala Met Ile Ile Ser Thr					
172	181	190	199	208	217
TCA CTT ATA ATT ACA GCC ATC ATA TTC ATA GCC TCG GCA AAC CAC AAA GTC ACA					
Ser Leu Ile Ile Thr Ala Ile Ile Phe Ile Ala Ser Ala Asn His Lys Val Thr					
226	235	244	253	262	271
CTA ACA ACT GCA ATC ATA CAA GAT GCA ACA AGC CAG ATC AAG AAC ACA ACC CCA					
Leu Thr Thr Ala Ile Ile Gln Asp Ala Thr Ser Gln Ile Lys Asn Thr Thr Pro					
280	289	298	307	316	325
ACA TAC CTC ACT CAG GAT CCT CAG CTT GGA ATC AGC TTC TCC AAT CIG TCT GAA					
Thr Tyr Leu Thr Gln Asp Pro Gln Leu Gly Ile Ser Phe Ser Asn Leu Ser Glu					

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**FIG.2B**

334	343	352	361	370	379
ATT ACA TCA CAA ACC ACC ACC ATA ATA CTA GCT TCA ACA ACA CCA CGA GTC AAG TCA					
Ile Thr Ser Gln Thr Thr Ile Leu Ala Ser Thr Thr Pro Gly Val Lys Ser					
388	397	406	415	424	433
AAC CTG CAA CCC ACA ACA GTC AAG ACT AAA AAC ACA ACA ACC CAA ACA CAA					
Asn Leu Gln Pro Thr Thr Val Lys Thr Lys Asn Thr Thr Thr Gln Thr Gln					
442	451	460	469	478	487
CCC AGC AAG CCC ACT ACA AAA CAA CGC CAA AAC AAA CCA CCA AAC AAA CCC AAT					
Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn Lys Pro Asn					
496	505	514	523	532	541
AAT GAT TTT CAC TTC GAA GIG TTT AAC TTT GTA CCC TGC AGC ATA TGC AGC AAC					
Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Ser Asn					
550	559	568	577	586	595
AAT CCA ACC TGC TGG GCT ATC TGC AAA AGA ATA CCA AAC AAA AAA CCA CGA AAG					
Asn Pro Thr Cys Tripl Ala Ile Cys Lys Arg Ile Pro Asn Lys Lys Pro Gly Lys					
604	613	622	631	640	649
AAA ACC ACC ACC AAG CCT ACA AAA CCA ACC TTC AAG ACA ACC AAA AAA GAT					
Lys Thr Thr Lys Pro Thr Phe Lys Pro Thr Phe Lys Thr Lys Lys Asp					

## FIG.2C

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658	667	676	685	694	703
CTC AAA CCT CAA ACC ACT AAA CCA CCA AAG GAA GTA CCC ACC ACC AAG CCC ACA GAA					
Leu Lys Pro Gln Thr Thr Lys Pro Lys Glu Val Pro Thr Thr Lys Pro Thr Glu					
712	721	730	739	748	757
GAG CCA ACC ATC AAC ACC ACC AAA ACA AAC ATC ACA ACT ACA CTG CTC ACC AAC					
Glu Pro Thr Ile Asn Thr Thr Lys Thr Asn Ile Thr Thr Thr Leu Leu Thr Asn					
766	775	784	793	802	811
AAC ACC ACA GCA AAT CCA AAA CTC ACA AGT CAA ATG GAA ACC TTC CAC TCA ACC					
Asn Thr Thr Gly Asn Pro Lys Leu Thr Ser Gln Met Glu Thr Phe His Ser Thr					
820	829	838	847	856	865
TCC TCC GAA GGC AAT CTA AGC CCT TCT CAA GTC TCC ACA ACA TCC GAG CAC CCA					
Ser Ser Glu Gly Asn Leu Ser Pro Ser Gln Val Ser Thr Thr Ser Glu His Pro					
874	883	892	901	914	
TCA CAA CCC TCA TCT CCA CCC AAC ACA ACA CGC CGC CAG TAGTTATTAA AAAA					
Ser Gln Pro Ser Ser Pro Pro Asn Thr Thr Arg Gln .					

**FIG.3A**

CAC AAA GTC ACA CTA ACA ACT GCA ATC ATA CAA GAT GCA ACA AGC CAG ATC AAG	54
His Lys Val Thr Leu Thr Ala Ile Ile Gln Asp Ala Thr Ser Gln Ile Lys	18
AAC ACA ACC CCA ACA TAC CTC ACT CAG GAT CCT CAG CTT CGA ATC AGC TTC TCC	108
Asn Thr Thr Pro Thr Tyr Leu Thr Gln Asp Pro Gln Leu Gly Ile Ser Phe Ser	36
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AAT CTG TCT GAA ATT ACA TCA CAA ACC ACC ATA CTA GCT TCA ACA ACA CCA	162
Asn Leu Ser Glu Ile Thr Ser Gln Thr Thr Ile Leu Ala Ser Thr Thr Pro	54
GCA GTC AAC TCA AAC CTG CAA CCC ACA ACA GTC AGT ACT AAA AAC ACA ACA ACA	216
Gly Val Lys Ser Asn Leu Gln Pro Thr Val Iys Thr Lys Asn Thr Thr Thr	72
ACC CAA ACA CAA CCC ACT ACA AAA CAA CGC CAA AAC AAA CCA CCA CCA	270
Thr Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro	90
AAC AAA CCC AAT AAT GAT TTT CAC TTC GAA GTG TTT AAC TTT GTA CCC TGC AGC	324
Asn Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser	108
ATA TGC AGC AAC AAT CCA ACC TGC TGG GCT ATC TGC AAA AGA ATA CCA AAC AAA	378
Ile Cys Ser Asn Asn Pro Thr Cys Trip Ala Ile Cys Lys Arg Ile Pro Asn Lys	126
AAA CCA CGA AAG AAA ACC ACC CCT ACA AAA AAA CCA ACC TTC AAG ACA	432
Lys Pro Gly Lys Lys Thr Thr Lys Pro Thr Lys Pro Thr Phe Lys Thr	144

**FIG.3B**

ACC AAA GAA GAT CTC AAA CCT CAA ACC ACT AAA CCA AAG GAA GTC CCC ACC ACC	486
Thr Lys Lys Asp Leu Lys Pro Gln Thr Thr Lys Pro Lys Glu Val Pro Thr Thr	162
AAG CCC ACA GAA GAG CCA ACC ATC AAC ACC AAA ACA AAC ATC ACA ACT ACA	540
Lys Pro Thr Glu Glu Pro Thr Ile Asn Thr Thr Lys Thr Asn Ile Thr Thr Thr	180
CTG CTC ACC AAC ACC ACA GGA AAT CCA AAA CTC ACA AGT CAA ATG GAA ACC	594
Leu Leu Thr Asn Asn Thr Thr GLY Asn Pro Lys Leu Thr Ser Gln Met Glu Thr	198
TTC CAC TCA ACC TCC TCC GAA GCC AAT CTA AGC CCT TCT CAA GTC TCC ACA ACA	648
Phe His Ser Thr Ser Ser Glu GLY Asn Leu Ser Pro Ser Gln Val Ser Thr Thr	216
TCC GAG CAC CCA TCA CAA CCC TCA TCT CCA CCC AAC ACA ACA CGC CAG TAG	699
Ser Glu His Pro Ser Gln Pro Ser Ser Pro Pro Asn Thr Thr Arg Gln	232
TTATTA AAAAATAA	

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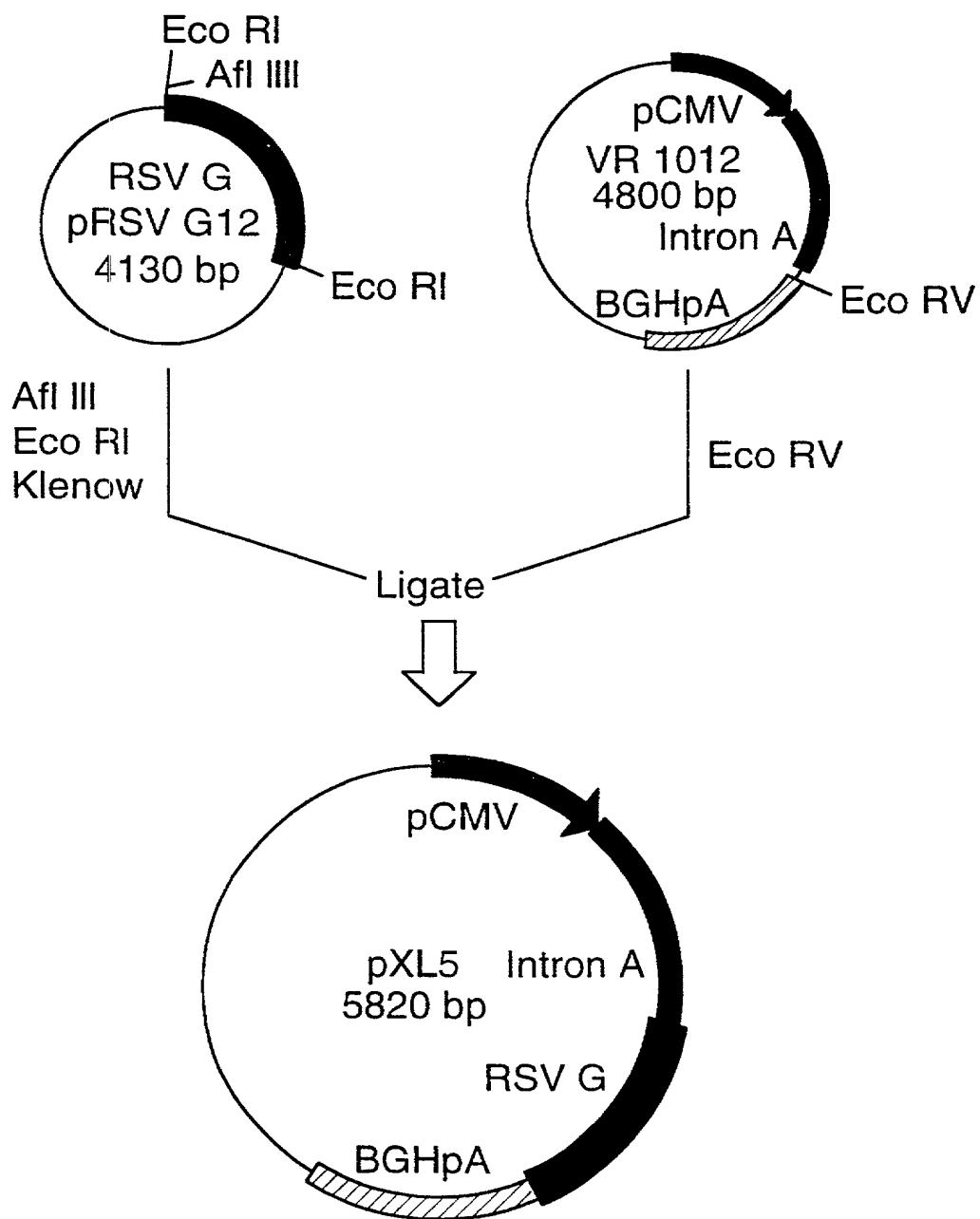


FIG.4.

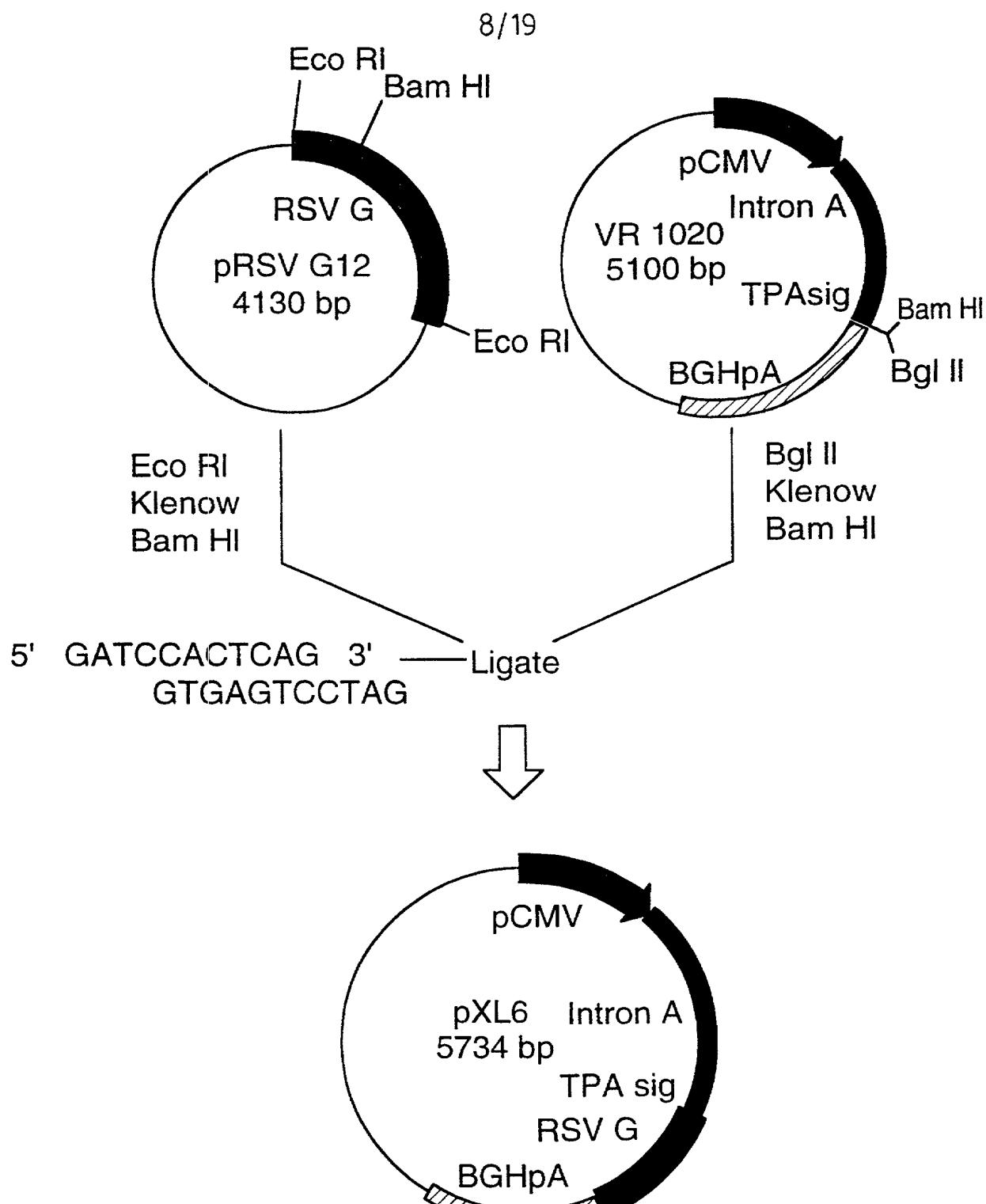


FIG.5

## FIG. 6A

10	20	30	40	50	60	70
TGGCGGTT CGGTGATGAC GGTGAAACC TCTCACACAT GCAGCTCCCG GAGACCGTCA CAGCTTGCT						
80	90	100	110	120	130	140
GTAAGCGCAT GCGGGAGGA GACAAGCCCG TCAGGGCGG TCAGGGGGG TTGGCGGTG TGGGGCTGG						
150	160	170	180	190	200	210
CTTAACATAG CGGCATCACA GCACATTTGA CTGAGAGHTGC ACCATATGCC GTGTGAAATA CGGCCACAGAT						
220	230	240	250	260	270	280
GGGTAAAGG AAAATAACCGC ATCAGATTGG CTATTGCCA TTGGATAAGT TGTATCCATA TCATAATATG						
290	300	310	320	330	340	350
TACATTATA TTGGCTCATG TCCAACATTA CGGCCATGT GACATTGATT ATTGACTAGT TATTAAATGT						
360	370	380	390	400	410	420
AATCAATTAC GGGTCATTAA GTTCATAGCC CATATATGGA GTTCGGCGTT ACATAACTTA CGGTAAATCG						
430	440	450	460	470	480	490
ccccccctggc TCAACCGCCA ACCAACCCCG CCCATTGACG TCAATAATGA CGTATGTTCC CATTAGTAACG						
500	510	520	530	540	550	560
CCAATAGGA CTTTCCATTG ACTGCAATGG GTGGAGTATT TACGGTAAAC TCCCCACTTG GGAGTACATC						

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**FIG. 6B**

570	580	590	600	610	620	630
AAGTGTATCA	TATGCCAAGT	ACGCCACCTA	TTCACGTCAA	TGACGGTAAA	TGGCCCGGCT	GGCATTTATGC
640	650	660	670	680	690	700
CCAGTACATG	ACCTTATGGG	ACTTTCCTAC	TTCGGCAGTAC	ATCTAACGTAT	TAGTCATCGC	TATTACCATG
710	720	730	740	750	760	770
GIGATGCCGT	TTTGGCAGTA	CATCAATCGG	CGTGGATAGC	GGTTTGACIC	ACGGGGATT	CCAAGTCTCC
780	790	800	810	820	830	840
ACCCCATTGA	CGTCAATCGG	AGTTTGTITTT	GGCACCAAAA	TCAACGGGAC	TTCACAAAT	GTGCGAACAA
850	860	870	880	890	900	910
CTCCCCCCA	TTCACGAAA	TCCCCTCTAG	CGCTGTACCG	TGGGAGGTCT	ATATAAGCAG	AGCTCGTTA
920	930	940	950	960	970	980
GTGAAACCGTC	ACATCGCTG	GAGACCCAT	CCACGGTGT	TTCGACTCCA	TAGAAGACAC	CGGGACCCGAT
990	1000	1010	1020	1030	1040	1050
CCAGGCTCTCG	CGCCCGGAA	CGGTGCAATG	GAACGGGGAT	TCCCGTGCCT	AAGAGTGAAC	TAAGTACCGC
1060	1070	1080	1090	1100	1110	1120
CTATAGACTC	TATAGGACA	CCACCTTGGC	TCTTATGCA	GCTTAACTGT	TTTTCGCTTG	GGCCCTATAC

## FIG. 6C

1130 1140 1150 1160 1170 1180 1190  
 ACCCCCCCTT CCTTATGCTA TAGGTGATGG TATAAGCTTAG CCTATAGCTG TCGGTTATGT ACCATTATG  
 1200 1210 1220 1230 1240 1250 1260  
 ACCACTCCCC TATGGTCAC GATACTTTC ATTACTAAC CATAACATGG CTCTTGCCA CAACTATCTC  
 1270 1280 1290 1300 1310 1320 1330  
 TATTEGCTAT ATGCCAATTAC TCTGTCCTTC AGAGACTGAC ACGGAACCTCTG TATTTTACA GGATGGGGTC  
 1340 1350 1360 1370 1380 1390 1400 11/19  
 CCATTATAA TTACAAATT CACATATACA ACAAGCCGT CCCCGGCCG OCAGTTTTT ATTAAACATA  
 1410 1420 1430 1440 1450 1460 1470  
 GGTGGGATC TCCACGGAA TCTGGGTAC GTGTTCGGGA CATGGCTCT TCTCCGGTAG CGGGGGGACT  
 1480 1490 1500 1510 1520 1530 1540  
 TCCACATCG AGCCCTGTC CCATGGCTCC ACCGGCTCAT GGTGGCTCGG CAGCTCTTG CTCTTAACAG  
 1550 1560 1570 1580 1590 1600 1610  
 TGAGGCCAG ACTTGGCAC AGCACAAATGC CCACCCACCAC CAGTGTGG CACAAGGCG TGGCGTAGG  
 1620 1630 1640 1650 1660 1670 1680  
 GTATGCTT GAAAATGAGC GTGGAGATTC GCTCTGGCAG GCTGACGGAG ATGGAAGACT TAAGGCAAGG

## FIG. 6D

1690	GCAGAAGAAG	ATGCCGGCAG	CIGAGTTGTT	GTATTCTCAT	AAGAGTCACA	GGTAACCTCC	GTGCGGGTGC	1750
1760	TGTTAACCGT	GGAGGGCAGT	GTAGTCTGAG	CAGTACTCTG	TCTTGCGCG	CGGCCACCA	GACATAATTAG	1820
1830	CTGACAGACT	AACAGACTGT	TCTCTTCAT	GGGTCTTTC	TCCAGTCACC	GTGCGGACA	CGTGTGATCA	1890
1900	CATATCCCG	CCGCTCTAGA	CCAGGCCCT	GAATCCAGAT	CTGCTGTTGCC	TCTTAGTTGC	CAGCCATCTTG	1960 12/16
1970	TCTTCCCC	CTCCCCCGTG	CCTTCCTTGA	CCCTGGAAAGG	TCCCCTACTCC	ACTGTCCTT	CTTAATAAAA	2030
2040	TGAGGAATT	GCATGCATT	GTCTGAGTAG	GTGTCATCT	ATTCTGGGG	GTGGCGGG	CCAGGACAGC	2100
2110	AAAGCCCCAGG	ATTCGGAGA	CAATACCAAGG	CATCTCTGG	ATGCCGTGG	CTCTATGGT	ACCCAGGTGC	2170
2180	TCAAGAATTG	ACCCGGTTCC	TCTTGGCCA	GAAGAAGCA	GCCACATCCC	CTTCCTCTTG	ACACACCCCTG	2240

## FIG. 6E

2250	2260	2270	2280	2290	2300	2310
TCCAGGCC	TTGTTCTTAG	TTCCAGCCCC	ACTCATAGGA	CACTCATAGC	TCAAGGGGC	TCCGGCTICA
2320	2330	2340	2350	2360	2370	2380
ATCCACCG	CTAAAGTACT	TGGAGGGTC	TCTCCCTCCC	TCATCAGGCC	ACCAAACCAA	ACCTAGCCTC
2390	2400	2410	2420	2430	2440	2450
CAAGAGTGG	AAGAAATTAA	AGCAAGATAG	GCTTATTAGT	GCACAGGGAG	AGAAAATGCC	TCCAACATGT
2460	2470	2480	2490	2500	2510	2520
GAGGAAGTAA	TGAGAGAAAT	CATAGAATT	CTTCCCGCTC	CTCGCTCACT	GACTCGCTGC	CTCGGGTGT
2530	2540	2550	2560	2570	2580	2590
TGGCTGGGG	CGAGGGGTAT	CAGCTCACTC	AAAGGCCGTA	ATACGGTTAT	CCACAGAAC	ACGGGATAAAC
2600	2610	2620	2630	2640	2650	2660
GCAGGAAAGA	ACATGTGAGC	AAAAGGCCAG	CAAAAGGCCA	GGAACCGTAA	AAAGGCCGG	TTGCTGGCGT
2670	2680	2690	2700	2710	2720	2730
TITTCATAG	GCTCCGCC	CCTGAGGAGC	ATCACAAAAA	TCCACGCICA	AGTCAGAGT	GGCCAAACCC
2740	2750	2760	2770	2780	2790	2800
GACAGGACTA	TAAAGATACC	CCCTGGAAAGC	TCCCTCTGTC	GCTCTCTGTC	TCCGACCCCTG	

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**FIG. 6F**

2810	2820	2830	2840	2850	2860	2870
CCGCTTACCG	GATACTGTC	CCCTTCTC	CCTTCGGAA	GGCTGGGCT	TTCTCATAGC	TCACGGTGTGA
2880	2890	2900	2910	2920	2930	2940
GGTATCTCAG	TTCGGTGTAG	GTCGGTGTGCT	CCAAGCTGG	CTGTGTGCAC	GAACCCCCCG	TTCAGCCCCGA
2950	2960	2970	2980	2990	3000	3010
CCGCTGCC	TTATCCGTA	ACTATGCT	TGAGTCCAAC	CCGGTAAAGAC	ACGACTTATC	CCCACTGGCA
3020	3030	3040	3050	3060	3070	3080 <sup>14</sup> / <sub>19</sub>
GCAGCCACTG	GTAACAGGAT	TAGCAGAGCG	AGGTATGTAG	GGGGTCTAC	AGAGTCTCTG	AAGTGGTGGC
3090	3100	3110	3120	3130	3140	3150
CTAACCTACG	CTACACTAGA	AGAACAGTAT	TGGTATCTG	CGCTCTCTG	AAGCCAGTTA	CTTGGAAA
3160	3170	3180	3190	3200	3210	3220
AAGAGTTGT	AGCTCTTGAT	CCGGCAAAACA	AACCACCGT	GGTAGGGGTG	TTTTTTTGT	TTGCAAGCAG
3230	3240	3250	3260	3270	3280	3290
CAGATTACGC	GCAGAAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTC	TACGGGTCT	GACGGCTCAGT
3300	3310	3320	3330	3340	3350	3360
GGAAACGAAA	CTGACGGTAA	GGGATTGG	TCATGAGATT	ATCAAAAGG	ATCTTCACCT	AGATCCCTT

## FIG.6G

3370	3380	3390	3400	3410	3420	3430
AAATTAAAAA	TCAAGTTTA	AATCAAATCA	AAGTATATAT	GAGTAACIT	GGTCCTCACAG	TTACCAATGC
3440	3450	3460	3470	3480	3490	3500
TTAACATCG	AGGCACCTAT	CTCAGGGATC	TGTCTTATTTC	GTTCATCCAT	AGTTGCCCTGA	CTGGGGGGGG
3510	3520	3530	3540	3550	3560	3570
GGGGGGCTG	AGGTCTGCT	CGTCAAGAAG	GTGGTGGCTGA	CTCATACCAAG	GCCTGAATTG	CCCCATCATC
3580	3590	3600	3610	3620	3630	3640
CAGCCAGAAA	GTGAGGGAGC	CACGGTTGAT	GAGAGCTTGT	TGTAGGTGG	ACCAAGTTGT	GATTITGAAC
3650	3660	3670	3680	3690	3700	3710
TTTGTCTTG	CCACGGAAACG	GTCTGGTTG	TCGGGAAGAT	GGTGATCTG	ATCCCTAAC	TCAGCAAAAG
3720	3730	3740	3750	3760	3770	3780
TTCGATTAT	TCAACAAAGC	CCCGCGTCCCG	TCAAGTCAGC	GTAAATGCTCT	GCCAGTGTAA	CAACCAATTA
3790	3800	3810	3820	3830	3840	3850
ACCAATTGIG	ATTAGAAAAA	CTCATCGAGC	ATCAAATGAA	ACTGCAATT	ATTCAATACA	GGATTATCAA
3860	3870	3880	3890	3900	3910	3920
TACCATATT	TGAAAAAGC	CGTTCTGTA	ATGAAACCA	AAACTCACCG	AGGCAGCTCC	ATACCAATGCC

## FIG. 6H

3930	3940	3950	3960	3970	3980	3990
AATATCCTGG	TATCGGTCTG	CGATTCCGAC	TGTCACAAC	TCAAATACAAC	CTATAAATT	CCCCTCGTCA
4000	4010	4020	4030	4040	4050	4060
AAAATAAGGT	TATCAAAGTGA	GAATCACCA	TGAGTGCAGA	CTGAATCCCG	TGAGAATGGC	AAAAGCTTAT
4070	4080	4090	4100	4110	4120	4130
GCATTTCCTT	CCAGACTTGT	TCAACAGGCC	AGCCATTACG	CTCGCTCATCA	AAATCACTCG	CATCAACCAA
4140	4150	4160	4170	4180	4190	4200 <sup>16</sup>
ACCGTTATTC	ATTGGTGTATT	GCGCCTGAGC	GAGACGAAT	ACGGGATCGC	TGTAAAGG	ACAATTACAA <sup>19</sup>
4210	4220	4230	4240	4250	4260	4270
ACAGGAATCG	AATGCAACCG	GCGCAGGAAC	ACTGCCAGCG	CATCAACAAAT	ATTTCACCT	GAATCAGGAT
4280	4290	4300	4310	4320	4330	4340
ATTCTCTAA	TACCTGGAAAT	GCTGTTTTCG	CGGGCATCGC	AGTGGTGAGT	AACCATGCAT	CATCAGGAGT
4350	4360	4370	4380	4390	4400	4410
ACGGATAAAA	TGCTTGTATCG	TGGAAAGAGG	CATAAATTCC	GTCAAGCCAGT	TTAGTCTGAC	CATCTCATCT
4420	4430	4440	4450	4460	4470	4480
GTACATCAT	TGGCAACGGCT	ACCTTTCCCA	TGTTTCAGAA	ACAACTCTGG	CGCATCGGC	TCCCCATACA

## FIG.6I

4490	4500	4510	4520	4530	4540	4550
ATCGATAGAT	TGTCGCACCT	GATTGCCCGA	CATTATCGCG	AGCCCAATTAA	TACCCATATA	AATCAGGATC
4560	4570	4580	4590	4600	4610	4620
CATGTGGAA	TTTAATCGCG	GCCTCGAGCCA	AGACGTTTCC	CGTGTGAATAT	GGCTCATAAC	GTTCCTTGTA
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4630	4640	4650	4660	4670	4680	4690
TTACTGTTA	TGTAAGCAGA	CAGTTTATT	GTTCATGATG	ATATATTTTT	ATCTTGCGA	ATGTAACATC
4700	4710	4720	4730	4740	4750	4760
AGAGATTITG	AGACACAAACG	TGGCTTCCC	CCCCCCCCCA	TTATTCAGG	TTTATTCAGG	GTATATTGCT
4770	4780	4790	4800	4810	4820	4830
CATGAGCGGA	TACATATTG	AAATGTTA	GAANANTAA	CAAATAGCGG	TTTCGGGCAC	ATTTCGGCGA
4840	4850	4860	4870	4880	4890	4900
AAAGTGCCAC	CTGACGCTCTA	AGAAACCATT	ATTATCATGA	CATTAACTTA	TAAAAATTAGG	CGTATCAGGA
4910						
GGCCCTTTCG	TC					

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FIG. 7

10            20            30            40            50            60            70  
CTCCAGTCAC CGTCGTCGAC CAGAGCTGAG ATCCTACAGG AGTCCAGGGC TGGAGAGAAA ACCTCTCGGA

80            90            100          110          120          130          140  
GGAAAGGCAA GGAGCAAGGCC GTGAAATTAA CGGAGGCTGT GAAGCCAATCA TGGATGCAAT GAAGGAGGG

150          160          170          180  
CTCTCTGIG TGCTGCTGCT GTGCTGACCA GTCCTCGTT CCCCCAGC

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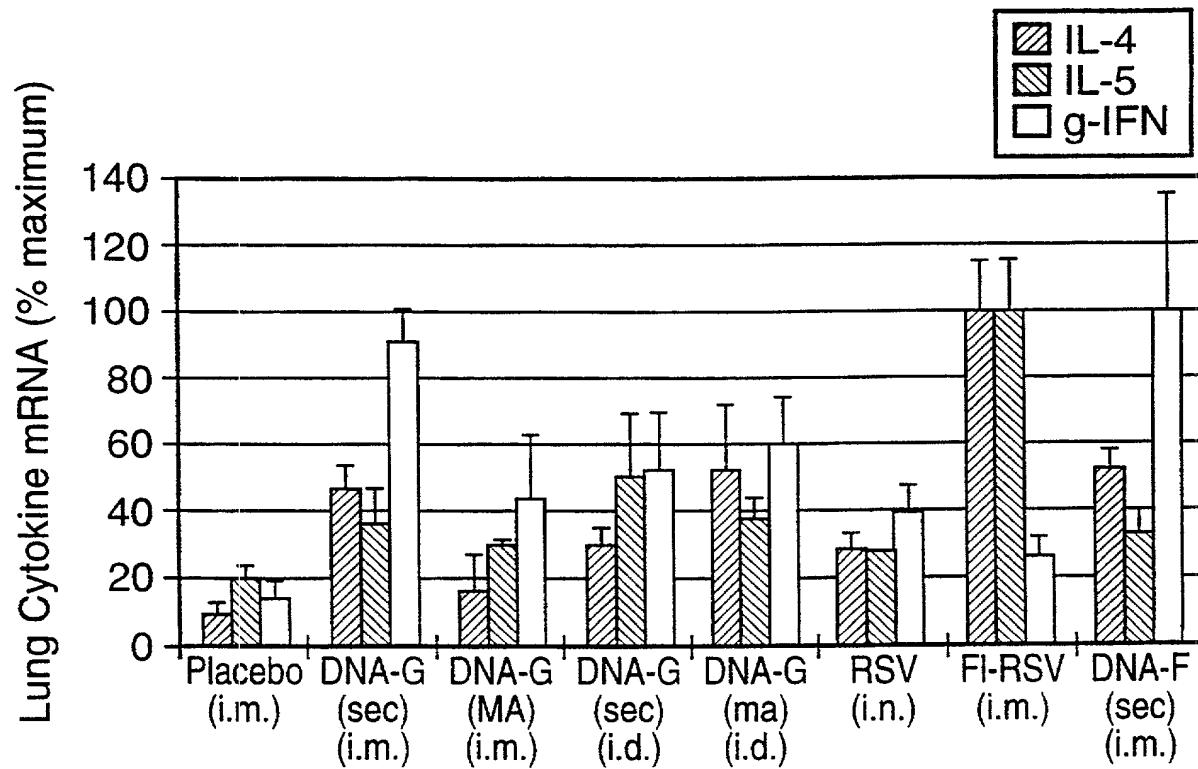
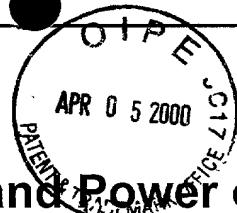


FIG.8



Docket No.  
1038-1003 MIS:jb

# Declaration and Power of Attorney For Patent Application

## English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

### NUCLEIC ACID VACCINES ENCODING G PROTEIN OF RESPIRATORY SYNCYTIAL VIRUS

the specification of which

(check one)

- is attached hereto.  
 was filed on July 16, 1998 as United States Application No. or PCT International Application Number PCT/CA98/00697  
and was amended on September 9, 1999

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

### Prior Foreign Application(s)

### Priority Not Claimed

(Number)	(Country)	(Day/Month/Year Filed)	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	<input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	<input type="checkbox"/>

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.) (Filing Date)

(Application Serial No.) (Filing Date)

(Application Serial No.) (Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

**08/896,442**

**July-18, 1997**

**Pending**

(Application Serial No.)

(Filing Date)

(Status)

(patented, pending, abandoned)

**PCT/CA98/00697**

**July 16, 1998**

(Status)

(Application Serial No.)

(Filing Date)

(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)

(patented, pending, abandoned)

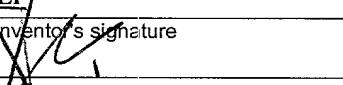
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

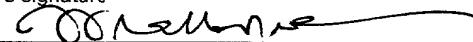
POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (*list name and registration number*)

Michael I. Stewart (24,973)

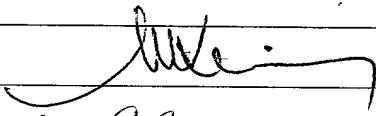
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Citizenship	
Post Office Address	

Full name of fifth inventor, if any	
Fifth inventor's signature	Date
Residence	
Citizenship	
Post Office Address	

Full name of sixth inventor, if any	
Sixth inventor's signature	Date
Residence	
Citizenship	
Post Office Address	